REMARKS

Status of the claims

Claims 21, 28, 43, 109-113, 120-135, and 137-143 were pending and claims 21 and 28 were under active examination. Claims 21 and 28 have been amended for clarity as shown above. Thus, claims 21, 28, 43, 109-113, 120-135, and 137-143 are pending as shown above and claims 21 and 28 are under active examination.

Claim Objections

Claims 21 and 28 were objected to for lack of clarity in reciting "chromosomal DNA." (Office Action, paragraph 2).

Applicants thank the Examiner for the careful attention to the claim language and have amended the claims as suggested, thereby obviating the objection.

35 U.S.C. § 103(a)

Claims 21 and 28 were again rejected under 35 U.S.C. § 103(a) as allegedly obvious over U.S. Patent Publication No. 2002/0107214 (hereinafter "Choulika") in view of Bibikova et al. (2001) *Mol. Cell. Biol.* 21:289-297 (hereinafter "Bibikova") and further in view of Takeuchi. (Office Action, paragraph 5). Choulika was cited for allegedly teaching the use of zinc finger proteins to repair a specific sequence of interest in chromosomal DNA of a cell. *Id.* Bibikova was cited for allegedly teaching the need for a pair of zinc finger nucleases. *Id.* Takeuchi was cited for teaching a vector comprising a Flp recombinase linked to a nuclear localization signal. *Id.*

Because there is no combination of the references that teaches the claimed vectors, Applicants traverse the rejection and supporting remarks.

It is axiomatic that the references must teach all the claimed elements. *In re Royka*, 490 F.2d 981, 985 (CCPA 1974). Furthermore, even if all the elements are present in the art, an obviousness rejection is only proper if "the improvement is no more than the predictable use of prior-art elements according to their established functions." *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007). Simply put, as stated by the Supreme Court, "there must be some articulated reasoning with some rational underpinning to support the

legal conclusion of obviousness." *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (quoting *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) (emphasis added)).

In the instant case, the claims clearly require a single vector that encodes two zinc finger nucleases that dimerize and cleave an endogenous gene. In addition, the vector also includes a repair substrate and a nuclear localization signal. The references do not teach all these elements and do not teach it is predictable to combine any of these elements as claimed.

As acknowledged, the primary reference (Choulika) fails entirely to teach or suggest a vector that encodes two zinc finger nucleases and/or includes a nuclear localization signal. (Office Action, page 4). However, Choulika also fails to teach or suggest cleavage of endogenous genes in the claimed cell types via delivery of polynucleotides encoding ZFEs with non-naturally occurring zinc finger domains. Rather, in all cases, Choulika uses SceI to cleave SceI target sites that are either episomal or artificially integrated into the genome. *See, e.g.,* Examples, particularly Example 3 of Choulika. Therefore, Choulika fails to teach or suggest vectors encoding two zinc finger nucleases, vectors including a nuclear localization signal as well as cleavage of an endogenous mammalian gene, as claimed.

Bibikova fails to supply the elements missing from Choulika, namely a vector comprising a repair substrate and a nuclear localization signal as well as sequences encoding at least two zinc finger nucleases for cleavage of an endogenous mammalian gene.

First and foremost, Bibikova fails to disclose a vector encoding at least two zinc finger nucleases. Instead, Bibikova discloses only microinjection of the protein form of chimeric nucleases. *See,* page 290, right column of Bibikova:

> **Enzymes**. Zif-QQR-F_N (29) and Zif-ΔQNK- F_N (51) were purified from over-producing bacteria ...
>
> **Oocyte injections.** ... the chimeric nuclease was in 10% glycerol was delivered to the nuclie in a volume of 2.5 to 15 nl. Two different QQR solutions were used for injections: one with an estimated concentration of 3 fmol/nl, and the other with 7 fmol/nl. ...

Thus, Bibikova fails entirely to teach or suggest any vector encoding at least two zinc finger nucleases.

Second, Bibikova fails to teach or suggest a vector including a repair substrate for an endogenous gene. Bibikova relates solely to local homologous recombination of the injected substrate (*see,* Figure 1B of Bibikova). In other words, this reference does not disclose a vector that includes a repair substrate. Rather, the target site and repair substrate are one and the same.

Third, Bibikova fails to disclose cleavage of an endogenous gene. As the target site (also repair substrate) is injected into the *Xenopus* oocyte, the target site is clearly not in an endogenous gene, as claimed. As such, Bibikova does not teach cleavage of endogenous genes.

Fourth, Bibikova also fails to teach ZFN-mediated cleavage in a mammalian cell. Bibikova injected exogenous substrates into *Xenopus* oocytes. Clearly, *Xenopus* oocytes are not mammalian cells. Nor are injected substrates in *Xenopus* oocytes in any predictive of endogenous genes in mammalian cells, as claimed. *Xenopus* oocytes are orders of magnitude larger than mammalian cells types. For example, the relative diameters of a *Xenopus* oocyte (1mm) and a HELA cell (~20 um) would indicate a fold volume difference of ~ 10^5. And, again, Bibikova relates only to cleavage of an injected substrate (which also serves as the repair substrate) following microinjection of protein forms of zinc finger nucleases. Simply put, microinjection of separate proteins into *Xenopus* oocytes to cleave an exogenously supplied substrate does not teach, suggest or in any predict the claimed vectors.

Finally, it is acknowledged that Takeuchi also fails to teach a vector comprising a repair substrate and sequences encoding at least two zinc finger nucleases.

Thus, there is no combination of Choulika, Bibikova and Takeuchi that teaches or suggests the claimed vectors. Choulika fails to teach or suggest a vector that encodes two zinc finger nucleases and/or includes a nuclease localization signal and cleavage of an endogenous gene. Bibikova fails to cure these deficiencies as it does not teach or suggest vectors encoding zinc finger nucleases (and therefore cannot teach vectors comprising both sequences encoding zinc finger nucleases and a repair substrate) for cleavage of

endogenous genes in mammalian cells. Moreover, the skilled artisan would not view the claimed vectors to be predictable based on any combination of Choulika's disclosure of SceI mediated cleavage of non-endogenous (integrated) targets and/or Bibikova's disclosure of protein microinjection to cleave an episomal target in *Xenopus* cells. In fact, because none of the references teaches cleavage of endogenous genes with zinc finger nucleases encoded on the same vector as a repair substrate, there are no circumstances in which combination of the cited references results in a vector as claimed.

CONCLUSION

For the reasons stated above, Appellants respectfully submit that the claims on appeal are in condition for allowance. Accordingly, Appellants request that the rejections of the claims on appeal be reversed, and that the application be remanded to the Examiner so that the appealed claims can proceed to allowance.

Respectfully submitted,

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